Genes encoding multiple forms of phospholipase A(2) are expressed in immature forms of human leukemic blasts.

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Letter to the Editor

Phospholipase A_2 (PLA_2) catalyzes the hydrolysis of the *sn*-2 position of membrane glycerophospholipids to liberate the eicosanoid precursor arachidonic acid (AA).[1,2] Three distinct families have been documented: low molecular weight soluble forms of PLA_2 ($sPLA_2$); Ca^{2+}-dependent high molecular weight PLA_2 ($cPLA_2$); and Ca^{2+}-independent high molecular weight PLA_2 ($iPLA_2$). The $sPLA_2$ family is implicated in several biological processes such as inflammation and host defense.[1,2] Nine isoenzymes have been identified. Among them $sPLA_2$-IB is the pancreatic one. $sPLA_2$-IIA is constitutively expressed in various organs related to immune response such as bone marrow spleen and thymus. $sPLA_2$-V is found in several immune cells such as macrophage, mast cells and type II helper T cells. $sPLA_2$-X is expressed in the digestive tract, immune organs and blood leukocytes. The $cPLA_2$ family consisted of four members, $cPLA_2$-IVA, $cPLA_2$-IVB, $cPLA_2$-IVC, $cPLA_2$-IVD; $cPLA_2$-IVA being the central regulator of stimulus-coupled cellular AA release.[1,2] The $iPLA_2$ (PLA_2-VI) plays a major role in phospholipids remodeling.

Eicosanoid products of the cyclooxygenase (COX) and lipoxygenase (LOX) pathways of AA are important mediators of malignant proliferation.[3] Deregulated PLA_2 activity contributes to the pathogenesis of several malignancies including prostate cancer, ovarian carcinoma and colorectal adenocarcinoma.[1,2] The COX and LOX pathways of AA have been recently documented on immature leukemic blasts of patients with acute myeloid (AML) and acute lymphoid (ALL) leukaemia.[4,5] Thus, freshly isolated AML and ALL blasts express COX-1, produce the AA metabolite prostaglandin E_2 (PGE_2), express

functional EP_2 receptors and increase their growth in response to exogenously added PGE_2. The World Health Organization (WHO) has proposed a classification system divides AML into several broad groups: AML with genetic abnormalities, AML with multilineage dysplasia, AML related to previous chemotherapy or radiation and AML not otherwise specified. The latter group contains a subgroup, AML without maturation, which consisted of the weaker immature blast group. In view of the potentially important oncogenic action of PLA_2 in leukemic proliferation, quantitative polymerase chain reaction (Q-PCR) was utilized to determine which of PLA_2 mRNAs were expressed in AML blasts without maturation and ALL blasts.

Blood samples recovered on EDTA were obtained from twenty one AML patients and ten B ALL patients at diagnosis according to the Helsinki recommendations. AML patients consisted in a homogeneous group of patients with blasts without signs of maturation and no genetic abnormality. Blood samples with more than 85% blast cells as circulating leukocytes were used. Leucocytosis ranged from 10 to 219 G/l. Immediately after recovery leukemic blasts were isolated by separation on a Ficoll gradient and washed one time with Hank's balanced salts solution (HBSS). The blast purity (>98%) was controlled by flow cytometry analysis. Blast viability (>95%) was judged by trypan blue exclusion. Blast RNA was immediately extracted with Tripure (Roche GmbH, Mannheim, Germany) and was stored at -80°C until used. As a control group, blood samples were recovered from 7 healthy volunteers. Control blood mononuclear cells were recovered and processed exactly as for leukemic blast samples.

In a first set of experiments we investigated if mRNAs derived from the four $cPLA_2$ genes were detected in AML and ALL leukemic blasts. As shown in Figure 1 (left part), mRNAs derived from three of the four cloned $cPLA_2$ genes are detected in human leukemic blasts. PLA_2-IVD transcripts were not present at detectable levels in AML and ALL

leukemic blasts and in blood mononuclear cells from healthy individuals (data not shown). In contrast, PLA_2-IVA, PLA_2-IVB and PLA_2-IVC were detected. Similar amounts of PLA_2-IVB and PLA_2-IVC transcripts were found in AML and ALL blasts and control cells. Both enzymes show little specificity for the *sn*-2 fatty acid.[1,2] In contrast, PLA_2-IVA transcripts were markedly ($p=0.006$) reduced in ALL blasts as compared with blood mononuclear cells. PLA_2-IVA preferentially hydrolyses phospholipids containing AA at the *sn*-2 position. PLA_2-IVA is ubiquitously and constitutively expressed in most cells and tissues. One notable exception is mature B and T cells which do not contain detectable levels of PLA_2-IVA.[1] Q-PCR analysis reveals that PLA_2-IVA levels were also markedly lowered in immature forms of ALL blasts. The high levels of AA documented in ALL blasts have been previously related to an elevated Δ^6 desaturase activity.[6] Low levels of PLA_2-IVA in ALL blasts might also explain these elevated AA amounts. PLA_2-VI ($iPLA_2$) transcript levels were higher ($p=0.003$) in AML and ALL leukemic blasts. PLA_2-VI was originally reported to mediate phospholipids remodelling and, thus, to act as a housekeeping protein without significant roles in cell growth.[1,2] Several recent studies have demonstrated that PLA_2-VI exhibited roles in cell regulation, growth and death. Especially, one mechanism by which PLA_2-VI mediates cell growth involves regulation of AA release, P53 and MAPK activation.[7] A role for PLA_2-VI may be suggested in the growth of AML and ALL blasts. The technique employed in this study allowed measurement of the absolute levels of any mRNA. So it was possible to evaluate the levels of each of the isoforms relative to one another. Results indicated the following rank of magnitude for $cPLA_2$ in leukemic blasts: PLA_2-VI > PLA_2-IVA = PLA_2-IVB > PLA_2-IVC. Together, these observations might suggest PLA_2-VI as a novel and interesting target for drug development for leukemic therapy. However, given the ubiquitous expression of PLA_2-VI and its role in glycerophospholipid metabolism, drug strategies targeting PLA_2-VI must exhibit selectivity

to avoid undesired side effects.

In another set of experiments we investigated if mRNAs derived from the nine sPLA$_2$ genes (*i.e* PLA$_2$-IB, PLA$_2$-IIA, PLA$_2$-IID, PLA$_2$-IIE, PLA$_2$-IIF, PLA$_2$-III, PLA$_2$-V, PLA$_2$-X and PLA$_2$-XII) were detected in AML and ALL leukemic blasts. PLA$_2$-IIE and PLA$_2$-III were not present at detectable levels in AML and ALL leukemic blasts and control blood mononuclear cells (data not shown). In contrast transcripts for other sPLA$_2$ subtypes could be detected (Figure 1, right part). Results indicated the following rank of magnitude for sPLA$_2$ in leukemic blasts: PLA$_2$-IB > PLA$_2$-XII > PLA$_2$-X > PLA$_2$-IID > PLA$_2$-IIA = PLA$_2$-V > PLA2-IIF. Levels of PLA$_2$-IB and PLA$_2$-X transcripts were higher ($p=0.0001$) in AML blasts than in control cells. In contrast PLA$_2$-IIA ($p=0.0005$), PLA$_2$-IID ($p=0.0007$) and PLA$_2$-V ($p=0.0009$) were markedly reduced while PLA$_2$-XII and PLA$_2$-IIF ones were unchanged. PLA$_2$-X transcripts were higher ($p=0.0006$) in ALL blasts than in control cells. In contrast PLA$_2$-IIA transcripts levels were reduced ($p=0.001$) while PLA$_2$-IB, PLA$_2$-IID, PLA$_2$-IIF, PLA$_2$-V and PLA$_2$-XII ones were unchanged. Such variations of PLA$_2$ subtypes would be of importance for leukemic patients. Thus, coagulation activation was often observed in patients with acute leukaemia. PLA$_2$-IIA, PLA$_2$-IID and PLA$_2$-V, which possess potent anticoagulant activity,[1] are markedly lowered in AML patients suggesting a putative link between PLA$_2$ activity and coagulation disorders. Bacterial and fungal infections are the major cause of morbidity and mortality in acute leukemic patients. A decreased PLA$_2$-IIA activity which has physiologically significant bactericidal activity,[1,2] and a decreased PLA$_2$-V one that plays a role in innate immunity against fungal invasion[1,2] might be implicated in these infections. Numerous evidences have highlighted that cytosolic PLA$_2$-IVA is the key enzyme for AA release from phospholipids of mammalian cells; AA being the first step for the biosynthesis of eicosanoids. Only those sPLA$_2$ species that have a high specific activity of phospholipids hydrolysis and that can bind well to

phosphatidylcholine-rich membranes, the PLA_2-V and PLA_2-X, have the capacity to release fatty acids when added to mammalian cells. As shown in Figure 1, the increase of PLA_2-X transcripts in leukemic blasts is higher than the decrease of their PLA_2-V transcripts. Thus, as lipolytic enzyme, PLA_2-X might contribute to the generation of lipid mediators. Of interest PGE_2, a COX metabolite of AA, was recently reported to stimulate the growth of leukemic blasts through and EP_2 receptor dependent pathway.[4]

These results demonstrate that mRNA from four out of five cytosolic PLA_2 (PLA_2-IVA, PLA_2-IVB, PLA_2-IVC and PLA_2-VI) and six out of nine $sPLA_2$ (PLA_2-IB, PLA_2-IIA, PLA_2-IID, PLA_2-V, PLA_2-X and PLA_2-XII) are present in leukemic blasts and that their mRNA transcript levels exhibited important variations as compared to blood mononuclear cells (summarized in Table I). One of the more notable finding is that leukemic blasts expressed high amounts of PLA_2-VI and PLA_2-X. This could be extremely significant as these two enzymatic activities play a major role in AA release for the generation of COX and LOX derived lipid mediators. In conclusion these results indicate that immature forms of leukemic AML and ALL blasts have the potential to express multiple isoforms of $cPLA_2$ and $sPLA_2$ which could be of importance given the potential role of these enzyme activities in inflammation, generation of lipidic mediators, anticoagulant activity and bacterial infection. Investigation of PLA_2 transcripts in other AML such as AML with genetic abnormalities and with multilineage dysplasia deserve now to be investigated.

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Legend to Figure 1

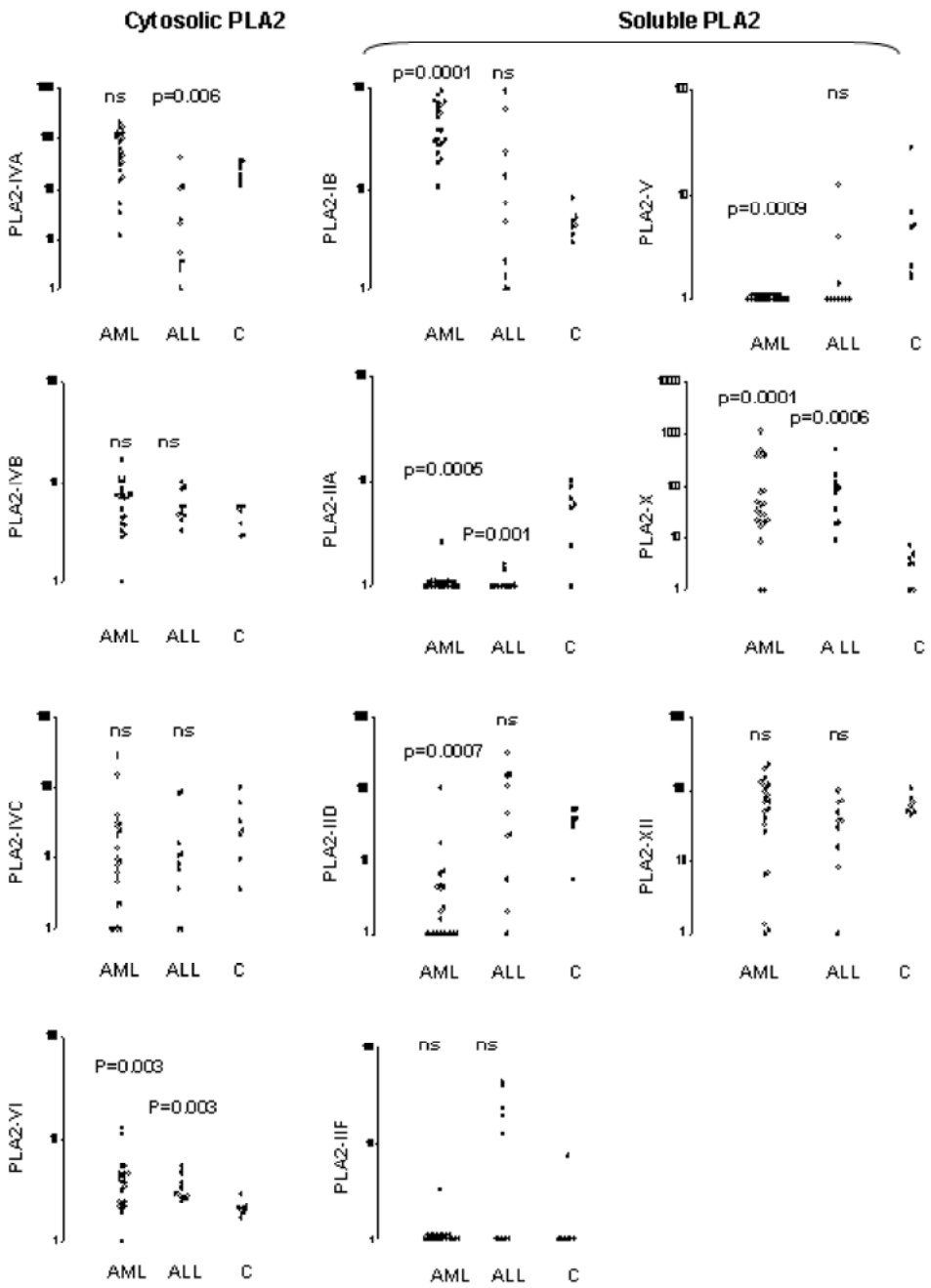
Q-PCR analysis of cytosolic PLA_2 and soluble PLA_2 transcripts in leukemic blasts.

Q-PCR was performed in duplicate by using TaqMan assay reagents according to the manufacturer's recommendations (Applied Biosystems, Foster City, CA) (product references - PLA_2-IB: Hs00386701-m1; PLA_2-IIA: Hs00179898-m1; PLA_2-IID: Hs00173860-m1; PLA_2-IIF: Hs00224482; PLA_2-IVA: Hs00233352-m1; PLA_2-IVB: Hs00979952-m1; PLA_2-IVC: Hs00234345-m1; PLA_2-IVD: Hs00603557-m1; PLA_2-V: Hs00173472-m1; PLA_2-VI: Hs001/85926-m1; PLA_2-X: Hs00358567-m1; PLA_2-XII: Hs00830106-s1). Gene expression levels were normalized to 18S RNA (product reference Hs99999901-s1). Amounts of various transcripts were compared to sample with the lowest level of transcripts (a patient who was arbitrary quoted 1). (●) indicates patients with no detectable transcript. Significance was assessed by using the Kruskal-Wallis test followed by a Mann-Whitney *U*-test. A Bonferroni adjustment indicated a $p<0.016$ as significant.

Table 1

	Soluble PLA_2									Cytosolic PLA_2				
	IB	IIA	IID	IIE	IIF	III	V	X	XII	IVA	IVB	IVC	IVD	VI
Controls	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
ALL	↔	↓	↔	No	↔	No	↔	↑	↔	↓	↔	↔	No	↑
AML	↑	↓	↓	No	↔	No	↓	↑	↔	↔	↔	↔	No	↑

Comparison of soluble PLA_2 and cytosolic PLA_2 transcripts levels in ALL blasts, AML blasts and control blood mononuclear cells. “Yes” and “No” indicated the presence and the absence of transcripts, respectively. “↔,↑,↓” indicated similar, elevated and decreased levels of transcripts as compared to blood mononuclear cell, respectively.


Cytosolic PLA2
Soluble PLA2
PLA2-IVA
ns
p=0.006
AML
ALL
C
PLA2-IB
p=0.0001
ns
PLA2-V
ns
p=0.0009
PLA2-IVB
ns
ns
PLA2-IIA
p=0.0005
P=0.001
PLA2-X
p=0.0001
p=0.0006
PLA2-IVC
ns
ns
PLA2-IID
p=0.0007
ns
PLA2-XII
ns
ns
PLA2-VI
P=0.003
P=0.003
PLA2-IIF
ns
ns